FORM PTO-1390 (REV. 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: 52.US2.PCT

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/807507

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR99/02517	October 15, 1999	October 15, 1998

TITLE OF INVENTION: SONDES FLUORESCENTES DE PEINTURE CHROMOSOMIQUE

APPLICANT(S) FOR DO/EO/US: Dorra CHERIF

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [x] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [x] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [x] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [x] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [x] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [x] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [x] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [x] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [ ] Other items or information:

09807507 , 052902
JC02 Rec'd PCT/PTO 13 APR 2001

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/807507	INTERNATIONAL APPLICATION NO. PCT/IB99/01730	ATTORNEY'S DOCKET NUMBER

21. ☐ The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	15 - 20 =	0	x **$18.00**	$ 0.00	
Independent claims	5 - 3 =	2	x **$80.00**	$ 160.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1,150.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. +				$ 0.00	
SUBTOTAL =				$ 1,150.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$ 0.00	
TOTAL NATIONAL FEE =				$ 1,150.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 0.00	
TOTAL FEES ENCLOSED =				$ 1,150.00	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $ 1,150.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 50-1181 in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1181 . A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

GENSET CORP.
10665 Sorrento Valley Road
San Diego, CA 92121-1609

Telephone: 858/597-2600
Facsimile: 858/597-2601

SIGNATURE

John Lucas, Ph.D., J.D.
NAME

43,373
REGISTRATION NUMBER

Express Mail Label #EL821903780US

Our Ref. No. 52.US2.PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:					
Inventor(s)	:	Dorra Cherif	Art Unit	:	Unassigned
Serial No.	:	09/807,507	Examiner	:	Unassigned
Filed	:	**October 15, 1999**			
Title	:	SONDES FLUORESCENTES DE PEINTURE CHROMOSOMIQUE			

SEQUENCE SUBMISSION STATEMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

A copy of the Sequence Listing in computer readable form on CD-ROM as required by 37 C.F.R. § 1.52(e), § 1.77(b)(4), § 1.821(e) and/or § 1.96(c) is submitted herewith.

The machine format is IBM-PC, the operating system is MS-Windows, the files on the CD-ROMs are SEQLIST.doc, SEQLIST.txt and README.txt and they were created on May 24, 2002. The size of the files is 50 kb.

The content of the sequence listing information recorded in computer readable form is identical to the written sequence listing and includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1,821(g), 1.825(b) or 1.825(d).

Respectfully submitted,

Dated: 5/24/02

By: Aaron Scalia

Aaron Scalia
Genset Corporation
10665 Sorrento Valley Road
San Diego, CA 921217
(858) 597-2600

Our Ref. No. 52.US2.PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:		
Inventor(s) : **Dorra CHERIF**	Art Unit	: Unknown
Serial No. :	Examiner	: Unassigned
Filed : **April 13, 2001**		
Priority Claimed on PCT Application:		
Serial No. : PCT/FR99/002517		
International Filing Date : October 15, 1999		
Title : SONDES FLUORESCENTES DE PEINTURE CHROMOSOMIQUE		

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the issuance of a first Office Action in the above-identified application, please amendment the application as follows:

In the Claims:

Please cancel claims 1-10 and 12-26 as filed in the PCT parent application upon which priority for this application is claimed.

Please amend claim 11, and add new claims 27-40, as follows.

11. (Amended) [Method] A method of producing probes intended for labeling human chromosomes[, characterized in that said method comprises the] comprising mixing [of two] first amplification products and second amplification products obtained by two IRS-PCR amplifications from said chromosomes [using, on the one hand,] wherein said first amplification products are obtained using PCR primers specific for the Alu and LINE DNA sequences, [and, on the other hand] said second amplification products are obtained using PCR primers specific for the Alu DNA sequences.

27. (Add) A method of identifying human chromosomes comprising performing a multicolor FISH analysis using a plurality of probes, said probes comprising a set of DNA segments which are more represented in certain chromosome bands and which are obtained by IRS-PCR amplification from said chromosomes with the aid of primers specific for the Alu and LINE DNA sequences.

28. (Add) The method of Claim 27, wherein the DNA probes are labeled with one or more fluorophores, each of said one or more fluorophores having a specific absorption and emission wavelength, wherein each of said one or more fluorophores is used with a pair of optical filters, one for absorption and one for emission, and wherein said fluorophores and pairs of filters are selected from the group consisting of:

(a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm used with the excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm used with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm used with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical);

(d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm used with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

(e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm used with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical);

(f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm used with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical);

(g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation

filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

29. (Add) The method of claim 28, wherein the optical filters exhibit the following qualities:

they are of the 6-cavity type;

they have an ADI of 0°;

they have a tolerance $\lambda_o \pm 20\%$ of FWHM;

they have a tolerance on FWHM of $\pm$ 20% of FWHM;

they have an OD5 out-of-passband rejection of UV at 1200 nm;

they have a transmission curve $T \geq 50\%$ at λ_o.

30. (Add) The method of claim 29, wherein the optical filters exhibit, in addition, the following characteristics:

they have a centered useful diameter greater than 21 nm;

they have a thickness $\leq$ 7 mm.

31. (Add) The method of claim 27, wherein said multicolor FISH analysis is a karyotype analysis.

32. (Add) The method of claim 31, wherein said karyotype analysis is performed to detect chromosome rearrangements.

33. (Add) A collection of fluorophores for labeling biomolecules said combination of fluorophores comprising at least 3 fluorophores selected from the group consisting of fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7) and Bodipy 630/650.

34. (Add) The collection of fluorophores of claim 33, wherein said combination comprises at least 4 of the fluorophores selected from the group consisting of fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7) and Bodipy 630/650.

35. (Add) The collection of fluorophores of claim 34, wherein said collection comprises at least 5 fluorophores selected from the group consisting of fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7) and Bodipy 630/650.

36. (Add) The collection of fluorophores of claim 35, wherein said collection comprises fluorescein isothiocyanate (FITC) Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), and cyanine 7 (Cy7).

37. (Add) A kit comprising at least one fluorophore having a specific absorption and emission wavelength, said kit further comprising at least one pair of optical filters, said pair of optical filters comprising one absorption filter for detecting signals at said absorption wavelength and one emission filter for detecting signals at said emission wavelength, wherein

said at least one fluorophore, absorption filter, and emission filter are selected from the group consisting of:

(a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm is coupled with the excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical);

(d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

(e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical);

(f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical);

(g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

38. (Add) The collection of fluorophores of claim 33, further comprising reagents for performing a multicolor FISH analysis.

39. (Add) A method for detecting a biological constituent comprising labeling a biological constituent selected from the group consisting of polypeptides, antibodies, nucleic acids, phospholipids, fatty acids, sterol derivatives, membranes, organelles and biological macromolecules with a collection of fluorophores according to claim 33 and detecting signals from said collection of fluorophores.

40. (Add) The method of claim 39, wherein said detecting step comprises performing a multicolor FISH analysis.

Our Ref. No. 52.US2.PCT

REMARKS

Claim 11 remains in this application. Claims 1-10 and 12-26 have been canceled. Claims 27-40 have been added.

Attached hereto is a page showing all the claims as currently pending, which is entitled "**Pending Claims**."

The Commissioner is hereby authorized to credit any overpayment or charge any additional fees in connection with the filing of this Preliminary Amendment to our Deposit Account No. 50-1181.

Respectfully submitted,
GENSET CORPORATION

Date: 13 Apr , 2001

By: _____
John Lucas, Ph.D., J.D.
Registration No. 43,373

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Certificate of Mailing under 37 CFR 1.10

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I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10, on the Date of Deposit shown above, postage prepaid and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

_____
Signature

AARON J. SCALIA
Typed or Printed Name of Person Signing Certificate

VERSION WITH MARKINGS TO SHOW CHANGES MADE

11. (Amended) [Method] A method of producing probes intended for labeling human chromosomes[, characterized in that said method comprises the] comprising mixing [of two] first amplification products and second amplification products obtained by two IRS-PCR amplifications from said chromosomes [using, on the one hand,] wherein said first amplification products are obtained using PCR primers specific for the Alu and LINE DNA sequences, [and, on the other hand] said second amplification products are obtained using PCR primers specific for the Alu DNA sequences.

27. (Add) A method of identifying human chromosomes comprising performing a multicolor FISH analysis using a plurality of probes, said probes comprising a set of DNA segments which are more represented in certain chromosome bands and which are obtained by IRS-PCR amplification from said chromosomes with the aid of primers specific for the Alu and LINE DNA sequences.

28. (Add) The method of Claim 27, wherein the DNA probes are labeled with one or more fluorophores, each of said one or more fluorophores having a specific absorption and emission wavelength, wherein each of said one or more fluorophores is used with a pair of optical filters, one for absorption and one for emission, and wherein said fluorophores and pairs of filters are selected from the group consisting of:

(a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm used with the excitation filter of the

490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm used with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm used with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical);

(d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm used with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

(e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm used with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical);

(f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm used with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical);

(g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

29. (Add) The method of claim 28, wherein the optical filters exhibit the following qualities:

they are of the 6-cavity type;

they have an ADI of 0°;

they have a tolerance $\lambda_o \pm 20\%$ of FWHM;

they have a tolerance on FWHM of ± 20% of FWHM;

they have an OD5 out-of-passband rejection of UV at 1200 nm;

they have a transmission curve $T \geq 50\%$ at λ_o.

30. (Add) The method of claim 29, wherein the optical filters exhibit, in addition, the following characteristics:

they have a centered useful diameter greater than 21 nm;

they have a thickness ≤ 7 mm.

31. (Add) The method of claim 27, wherein said multicolor FISH analysis is a karyotype analysis.

32. (Add) The method of claim 31, wherein said karyotype analysis is performed to detect chromosome rearrangements.

33. (Add) A collection of fluorophores for labeling biomolecules said combination of fluorophores comprising at least 3 fluorophores selected from the group consisting of fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7) and Bodipy 630/650.

34. (Add) The collection of fluorophores of claim 33, wherein said combination comprises at least 4 of the fluorophores selected from the group consisting of fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7) and Bodipy 630/650.

35. (Add) The collection of fluorophores of claim 34, wherein said collection comprises at least 5 fluorophores selected from the group consisting of fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7) and Bodipy 630/650.

36. (Add) The collection of fluorophores of claim 35, wherein said collection comprises fluorescein isothiocyanate (FITC) Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), and cyanine 7 (Cy7).

37. (Add) A kit comprising at least one fluorophore having a specific absorption and emission wavelength, said kit further comprising at least one pair of optical filters, said pair of optical filters comprising one absorption filter for detecting signals at said absorption wavelength and one emission filter for detecting signals at said emission wavelength, wherein said at least one fluorophore, absorption filter, and emission filter are selected from the group consisting of:

(a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm is coupled with the excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical);

(d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

(e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical);

(f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical);

(g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

38. (Add) The collection of fluorophores of claim 33, further comprising reagents for performing a multicolor FISH analysis.

39. (Add) A method for detecting a biological constituent comprising labeling a biological constituent selected from the group consisting of polypeptides, antibodies, nucleic acids, phospholipids, fatty acids, sterol derivatives, membranes, organelles and biological macromolecules with a collection of fluorophores according to claim 33 and detecting signals from said collection of fluorophores.

40. (Add) The method of claim 39, wherein said detecting step comprises performing a multicolor FISH analysis.

Claims as Pending after Preliminary Amendment

11. A method of producing probes intended for labeling human chromosomes comprising mixing first amplification products and second amplification products obtained by two IRS-PCR amplifications from said chromosomes wherein said first amplification products are obtained using PCR primers specific for the Alu and LINE DNA sequences, said second amplification products are obtained using PCR primers specific for the Alu DNA sequences.

27. (Add) A method of identifying human chromosomes comprising performing a multicolor FISH analysis using a plurality of probes, said probes comprising a set of DNA segments which are more represented in certain chromosome bands and which are obtained by IRS-PCR amplification from said chromosomes with the aid of primers specific for the Alu and LINE DNA sequences.

28. (Add) The method of Claim 27, wherein the DNA probes are labeled with one or more fluorophores, each of said one or more fluorophores having a specific absorption and emission wavelength, wherein each of said one or more fluorophores is used with a pair of optical filters, one for absorption and one for emission, and wherein said fluorophores and pairs of filters are selected from the group consisting of:

(a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm used with the excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm used with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm used with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical);

(d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm used with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

(e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm used with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical);

(f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm used with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical);

(g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation

filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

29. The method of claim 28, wherein the optical filters exhibit the following qualities:

they are of the 6-cavity type;

they have an ADI of 0°;

they have a tolerance $\lambda_o \pm 20\%$ of FWHM;

they have a tolerance on FWHM of $\pm$ 20% of FWHM;

they have an OD5 out-of-passband rejection of UV at 1200 nm;

they have a transmission curve $T \geq 50\%$ at λ_o.

30. (Add) The method of claim 29, wherein the optical filters exhibit, in addition, the following characteristics:

they have a centered useful diameter greater than 21 nm;

they have a thickness $\leq$ 7 mm.

31. The method of claim 27, wherein said multicolor FISH analysis is a karyotype analysis.

32. The method of claim 31, wherein said karyotype analysis is performed to detect chromosome rearrangements.

33. A collection of fluorophores for labeling biomolecules said combination of fluorophores comprising at least 3 fluorophores selected from the group consisting of fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7) and Bodipy 630/650.

34. The collection of fluorophores of claim 33, wherein said combination comprises at least 4 of the fluorophores selected from the group consisting of fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7) and Bodipy 630/650.

35. The collection of fluorophores of claim 34, wherein said collection comprises at least 5 fluorophores selected from the group consisting of fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7) and Bodipy 630/650.

36. The collection of fluorophores of claim 35, wherein said collection comprises fluorescein isothiocyanate (FITC) Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), and cyanine 7 (Cy7).

37. A kit comprising at least one fluorophore having a specific absorption and emission wavelength, said kit further comprising at least one pair of optical filters, said pair of optical filters comprising one absorption filter for detecting signals at said absorption wavelength and one emission filter for detecting signals at said emission wavelength, wherein

said at least one fluorophore, absorption filter, and emission filter are selected from the group consisting of:

(a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm is coupled with the excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical);

(d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

(e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical);

(f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical);

(g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

38. The collection of fluorophores of claim 33, further comprising reagents for performing a multicolor FISH analysis.

39. A method for detecting a biological constituent comprising labeling a biological constituent selected from the group consisting of polypeptides, antibodies, nucleic acids, phospholipids, fatty acids, sterol derivatives, membranes, organelles and biological macromolecules with a collection of fluorophores according to claim 33 and detecting signals from said collection of fluorophores.

40. The method of claim 39, wherein said detecting step comprises performing a multicolor FISH analysis.

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FLUORESCENT PROBES FOR CHROMOSOME PAINTING

The present invention relates to chromosome painting and more particularly the fluorescent probes which can be used in methods such as the FISH ("Fluorescence *In Situ* Hybridization") method. The invention also relates to combinations of fluorophores and of optical filters.

In situ hybridization is a technique which makes it possible to detect a DNA (or RNA) sequence by means of a probe having a specific sequence which is homologous to that studied. It is based on the complementarity of the nucleotides (A/T, A/U, G/C) and it can be carried out under precise physicochemical conditions on chromosome or tissue preparations. The result of the *in situ* hybridization process is the formation of a hybrid between a probe and a target. *In situ* hybridization includes a denaturing step and also a step for detecting the hybrid or the probe which is carried out after the *in situ* hybridization of the probe to the target. The sample may adhere in the form of a layer to the surface of the slide and this sample may, for example, comprise or contain individual chromosomes or chromosomal regions which have been treated in order to maintain their morphologies under denaturing conditions. In the context of fluorescence *in situ* hybridization, the probes are labeled with a fluorophore and the hybridization is revealed by fluorescent labeling.

The recent development of this technique allows the simultaneous visualization, on the same preparation, of several probes each revealed by a different fluorophore. This technique, called multicolor FISH or multi-FISH, has been made possible by the combination of filters specific for the wavelengths of emission of the different fluorescent molecules ensuring the labeling with the aid of a computer-aided imaging carried out by means of infrared-sensitive high-resolution cold CCD cameras (Schröck et al., 1996; Speicher et al., 1996).

The use of probes having a specific sequence homologous to a precise chromosomal sequence or a whole chromosome coupled with the potential for a multicolor fluorescent labeling makes it possible to develop so-called chromosome painting techniques, that is to say to obtain chromosomes of different colors and thus to obtain, if desired, a multicolor complete karyotype. Karyotype is understood to mean the characteristic arrangement of the chromosomes of a cell in the

metaphase.

Within the general meaning of the term, "labeling" is understood to mean an entity such as a radioactive isotope or a nonisotopic entity such as enzymes, biotin, avidin, steptavidin, digoxygenin, luminescent agents, dyes, haptens and the like. The luminescent agents, depending on the source of excitation energy, may be classified into radioluminescent, chemoluminescent, bioluminescent and photoluminescent (including fluorescent and phosphorescent) agents. The term "fluorescent" refers in general to the property of a substance (such as a fluorophore) to produce light when it is excited by an energy source such as ultraviolet light for example.

"Chromosomal paint probe" is understood to mean a probe or a probe composition such as the probe composition of this invention, which is suitable for hybridizing, under hybridization conditions, with a target which comprises a predetermined chromosome of a multichromosomal genome. If only a fraction of such a chromosome is present in the sample being subjected to such a hybridization with such a probe composition, then this fraction hybridizes and is identified. In practice, a painted probe of this invention may be mixed with a second, a third, and the like, so as to allow the labeling and the simultaneous detection of the two, three, and the like, predetermined chromosomes.

The visualization of all the 24 human chromosomes has been made possible by the use of a labeling with a combination of fluorochromes. For example, in the case of the use of 5 different fluorophores, 31 combinations of fluorophores can be obtained. By using this labeling principle and 24 DNA probes specific for each of the human chromosomes, it has been possible to visualize each chromosome differentially. The attribution, by computer processing, of artificial colors to each of the combinations of fluorophores thus makes it possible to color the 24 human chromosomes differently.

Rapidly, the strong potentials of such a multicolor labeling have allowed the analysis of chromosomal aberrations which were difficult to detect up until now by conventional cytogenetic techniques for labeling chromosomes in bands (Summer et al., 1971; Dutrillaux and Lejeune, 1971) (Giema staining, labeling with BrdU, and the like). The principle of labeling of chromosomes in bands is based on the

differences in the average base pair composition (richness in GC) between the bands and on the differences in the compaction of the chromatin between the chromosome bands. Chromosome painting has proved to be a very useful tool for detecting interchromosomal aberrations such as translocations, amplified DNA sequences such as the homogeneously stained regions called HSR (HSR for Homogeneously Staining Regions) or the excesses of chromosomal materials such as the marker chromosomes or double-minute chromosomes. Intrachromosomal aberrations such as deletions and duplications will only be detected as a function of the size of the aberrations, if the latter affect the length of the chromosomes, whereas chromosomal inversions will not at all be detectable by this method.

The limits of the use of the current spectral karyotyping as such are due to the fact that it does not make it possible to detect the nature of the chromosome bands involved in an inter- or intrachromosomal rearrangement. To do this, it is essential to couple this technique to the more conventional one of chromosome bands (R or G labeling) such as DAPI counterstaining, Giemsa or propidium iodide staining for example.

The requirement to combine different techniques of course constitutes a handicap in the analysis of chromosomal aberrations and, moreover, the use of the FISH or multi-FISH method which combines the high cost of the apparatus and the instrumentation necessary for the visualization of chromosome painting with the high cost of the probes specific for the chromosomes restricts the possibilities of this technique spreading to research laboratories or to diagnostic laboratories.

Paint probes currently available on the market (GIBCO-BRL, Oncor, Boehringer Manheim and the like) are obtained by DOP-PCR amplification using degenerate PCR primers of chromosomes or for fragments of chromosomes isolated by cumbersome techniques such as chromosome sorting by flow cytometry or microdissection of chromosomes. The hybridization of probes obtained by DOP-PCR does not generate chromosome bands on the chromosomes. The generation of chromosome bands was sought through the creation of artificial bands along chromosomes. This creation of artificial bands requires the use of cumbersome and expensive techniques. Furthermore, it results in bands which are not known reference marks in the field of cytogenetics.

Some others have described the use of chromosome paint probes obtained by amplification of chromosomes by IRS-PCR (Interspersed Repeated Sequences) using primers specific for DNA sequences which are repeated and dispersed in the genome, such as the Alu and LINE sequences. The combined use of LINE and Alu PCR primers for the amplification of human chromosomes by ISR-PCR was previously proposed by Lichter et al., 1990. However, the labeling in R bands which was obtained by the latter does not make it possible to ensure complete painting covering all the regions of the genome, in particular the telomeric regions and certain G chromosome bands.

The object of the present invention is to provide chromosomal probes which can be obtained inexpensively and which, in addition, make it possible to cause quality chromosome bands to appear directly on chromosomes painted in their entirety.

To do this, the present invention relates to probes intended for the labeling of a chromosome, characterized in that they are composed of a set of DNA segments which are more represented in certain chromosome bands and which are obtained by IRS-PCR amplification from said chromosomes with the aid of primers specific for the Alu and LINE DNA sequences.

The term "probe" refers to a polynucleotide or a mixture of polynucleotides such that DNA segments or DNA sequences are chemically combined with labeled individual entities. Each of the polynucleotides constituting a probe is characteristically in single-stranded form at the time of hybridization to the target.

The term "DNA fragment", "DNA segment" generally indicates only a portion of the polynucleotide or of a sequence present in a chromosome or a chromosome portion. A polynucleotide for example may be cut or fragmented into a multitude of segments or of fragments. A chromosome characteristically contains regions which have DNA sequences containing repeated DNA segments. The term "repeated" refers to the fact that a particular DNA segment is present many times (at least twice), dispersed or otherwise in the genome. The so-called IRS-PCR method using primers which hybridize with the dispersed repeated sequences of the genome, such as, for example, the Alu or LINE sequences.

"Genome" designates the complete and unique copy of the genetic

instructions of an organism encoded by the DNA of this organism. In the present invention, the particular genome considered is multichromosomal such that the DNA is distributed in the cell between several individual chromosomes. The human genome is composed of 23 pairs of chromosomes, of which an XX or XY pair determine the sex.

The term "chromosome" refers to the support for the genes carrying heredity in a living cell which is derived from chromatin and which comprises DNA and protein components (essentially histones). The conventional international system for identifying and numbering the chromosomes of the human genome is used here. The size of an individual chromosome may vary within a multichromosomal genome and from one genome to another. In the present case of the human genome, the total length of DNA of a given chromosome is generally greater than 50 million bp. By way of comparison, the total length of the human genome is 3×10^9 bp.

The genome of mammals contains repeated DNA sequences dispersed over the whole genome. In humans, the majority of this type of sequences is represented by the different families of Alu sequences, which are about 10^6 in number and have in common a consensus sequence of 300 bp. The repeated LINES (or L1) sequences are, like the Alu sequences, widely distributed over the whole genome. They are nevertheless less numerous (about 10^4). Their consensus sequence is about 6 kb. They are preferably situated in the dark G bands (positive G or negative R), whereas the Alu sequences are instead situated in the dark R bands (positive R) (Korenberg and Kirowski, 1988).

The DNA segments amplified by IRS-PCR according to the invention may have as source somatic hybrids, preferably rodent-human somatic hybrids, chromosomes or fragments of chromosome. The chromosomes or fragments of chromosome may be obtained by chromosome sorting by flow cytometry or by chromosome microdissection. Within the framework of the preferred embodiment of the present invention, the DNA segments amplified by IRS-PCR have as source mono-chromosomal rodent-human somatic hybrids.

The DNA segments amplified by IRS-PCR according to the invention are more represented in one type of cytogenetic band. The preferred cytogenetic bands

are the G bands or the R bands. In the preferred embodiment of the present invention, the DNA segments amplified by IRS-PCR are more represented in R bands.

Repeated sequences, analogous to the human repeated sequences, are also found in the genome of rodents. However, the divergence of this type of sequences between humans and rodents is sufficiently important for there to be little homology between them. This divergence allows a selective amplification of DNA segments contained in the human chromosome when human-rodent hybrids are used. Thus, starting with the DNA of a human-rodent somatic hybrid and using primers specific for the Alu and/or L1 consensus sequence, it is possible to selectively amplify by PCR the DNA sequences between two repeated sequences ("inverted" position) separated by a distance < 5 kb. The product of amplification thus obtained consists of a set of fragments (whose size varies approximately from 100 bp to 5 kb) which is representative of practically the entire human chromosome contained in the DNA of the somatic hybrid.

In general, the present invention is of course more particularly intended for producing probes specific for human chromosomes, although it is possible to envisage chromosome paintings for other cell types (Sabile et al., 1997).

The probes of the present invention are characterized in that the probes are derived from a mixture of two IRS-PCR amplification products which is composed of:

- PCR amplification product obtained using the primer specific for the Alu DNA sequences,

- PCR amplification product obtained using the primer specific for the Alu DNA sequences and the primer specific for the LINE DNA sequences.

The present invention relates to a method of producing probes intended for labeling human chromosomes, characterized in that said method comprises the mixing of two amplification products obtained by two IRS-PCR amplifications from said chromosomes using, on the one hand, PCR primers specific for the Alu and LINE DNA sequences, and, on the other hand, PCR primers specific for the Alu DNA sequences.

Any primer specific for the Alu or LINE sequences may be used in the

present invention. Preferably, the primers specific for the Alu DNA sequences consist of the SR1 primer whose sequence is described in SEQ ID No 1 and the primer specific for the LINE DNA sequence is preferably the L1H primer whose sequence is described in SEQ ID Nos 2 and 3.

Alternatively, probes according to the present invention may also be derived from a mixture of two IRS-PCR amplification products which is composed of:

- PCR amplification product obtained using the primer specific for the LINE DNA sequences,

- PCR amplification product obtained using the primer specific for the Alu DNA sequences and the primer specific for the LINE DNA sequences.

The present invention also relates to a method of producing probes intended for labeling human chromosomes, characterized in that said method comprises the mixing of two amplification products obtained by two IRS-PCR amplifications from said chromosomes using on the one hand PCR primers specific for the Alu and LINE DNA sequences, and, on the other hand, PCR primers specific for the LINE DNA sequences.

The present invention also comprises the use of fluorophores and of filters whose combination makes it possible to ensure a chromosome painting providing very readable karyotypes, that is to say to obtain contrasted and well-defined chromosome paint colors.

Thus, the DNA probes described above are labeled directly or indirectly by fluorescence techniques. Non-exhaustively, the fluorophores used for the labeling may be chosen from markers of the cyanine, rhodamine, fluorescein, Bobipy, Texas Red, Oregon Green, Cascade Blue type. In particular, all the fluorophores cited in "Handbook of fluorescent probes and research chemicals" (Richard P Haugland, 1996, Molecular Probes, MTZ Spence Ed., more particularly p 145-146, 153, 155-156, 157-158, 161) can be used to label the probes of the present invention.

Preferably, the probes according to the invention are labeled with at least 1, 2, 3, 4 or 5 fluorophores chosen from the following group: fluorescein isothiocyanate (FITC), Texas Red (TR for Texas Red), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

The preferred method for labeling the DNA probes is "Nick translation".

However, the labeling may also be carried out by all the standard reactions for synthesis of DNA catalyzed by a polymerase and for labeling oligonucleotides. For example, the labeling may be carried out by the techniques of random priming, amplification or primer extension in situ.

The term "directly labeled probe" designates or describes a nucleic acid probe whose labeling after the formation of hybrid with the target is detectable without subsequent reagent treatment of the hybrid. The probes using the FITC, Texas Red, Cy3 and Cy5 fluorophores according to the present invention are directly labeled.

The term "indirectly labeled probe" designates or describes a nucleic acid probe whose labeling after the formation of hybrid with the target must undergo an additional reagent treatment with one or more reagents in order to combine therewith one or more entities from which a detectable compound finally result(s). For example, the probes may be labeled with DNP, digoxigenin or biotin and the revealing comprises bringing the probe into contact with an anti-DNP or anti-digoxigenin antibody labeled with a fluorophore or with an avidin coupled to a fluorophore. The probes using the fluorophores Cy7, Bodipy 630/650, Cy5.5. according to the present invention are indirectly labeled.

Preferably, the probe composition of the present invention comprises the largest possible number of "directly labeled" probes. In addition to the fact that the directly labeled probes are easier to use, they allow better resolution. This good resolution is important for good observation of the chromosome bands. Preferably, the probe composition of the present invention is of the "direct labeling" type for all the fluorophores. A preferred probe composition of the present invention is of the "direct labeling" type for 4 of the 5 fluorophores used. In another preferred composition, the directly labeled probes represent 3 probes out of 5 or 6 fluorophores used.

The present invention also relates to a set of probes intended for labeling human chromosomes, characterized in that it contains probes according to the present invention for each of the human chromosomes and for a number of them. This set of probes will make it possible to analyze in a single operation a complete karyotype so as to detect therein and to identify therein possible chromosomal aberrations as described above.

The present invention also relates to a multicolor FISH method intended for studying the karyotype, characterized in that the DNA probes are labeled with fluorophores and in that each fluorophore having a specific absorption and emission wavelength is combined with a pair of optical filteres, one for absorption and one for emission.

The fluorophores are chosen such that the overlapping of the absorption and emission spectra between the different fluorophores is minimal. More particularly, it is important that there is no overlapping between the absorption and emission maxima of the different fluorophores.

Each fluorophore is used with a pair of optical filters; one for absorption and one for emission. The filters make it possible to select the passbands such that the wavelengths corresponding to an overlap with another fluorophore are eliminated. Accordingly, the filters used in the present invention are preferably of the narrow passband optical filter type. The filters are preferably of a superior quality because it is important that the filter does not allow light outside the passband to pass through.

Preferably, the present invention also relates to a multicolor FISH method intended in particular for studying the karyotype, characterized in that the DNA probes according to the present invention are labeled with fluorophores and in that each fluorophore having a specific absorption and emission wavelength is combined with a pair of optical filters, one for absorption and the other for emission, said method using fluorophores and pairs of filters chosen from the following group:

a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm combined with an excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical),

b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical),

c) the fluorophore TR having a maximum absorption wavelength of 593 nm

and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical),

d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emission wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical),

e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical),

f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical),

g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm combined with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650DF10 type (Omega Optical).

The invention also relates to a multicolor FISH diagnostic kit characterized in that it comprises DNA probes according to the present invention labeled with fluorophores and in that each fluorophore having a specific absorption and emission wavelength is combined with a pair of optical filters, one for absorption and one for emission, said kit using fluorophores and pairs of filters chosen from the following group:

a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm combined with an excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical),

b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10

type (Omega Optical),

c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical),

d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emission wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical),

e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical),

f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical),

g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm combined with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650DF10 type (Omega Optical).

The filters according to the present invention are preferably such that:

- they are of the 6-cavity type,
- they have an ADI of 0°;
- they have a tolerance $\lambda_0 \pm 20\%$ of FWHM,
- they have a tolerance on FWHM of $\pm$ 20% of FWHM,
- they have an OD5 out-of-passband rejection for UV at 1200 nm
- they have a transmission curve $T \geq 50\%$ at λ_0 .

Preferably, the filters must also have a centered useful diameter greater than 21 mm, and a thickness $\leq$ 7 mm,

The fluorophores and the above filters may be used for the labeling of the probes according to the present invention or alternatively for different probes used, for example, for chromosome painting or for multicolor FISH.

In the present invention, "filters" is understood to mean narrow passband interference filters which transmit light within a given very narrow spectral band centered around the reference wavelength λ_0. They are characterized by their transmission curve: $T = f(\lambda)$. The width of the band is defined by the full width at half maximum transmission (FWHM for "Full Width at Half Maximum transmission").

Outside the passband, the filter allows a residual signal which is as attenuated as possible to pass through.

The interference filters function on the principle of constructive and destructive interference. The basic component of an interference filter is called cavity. It has two stacks of reflectors separated by a layer of a dielectric solid. The higher the number of cavities, the more rectangular the shape of the transmission curve (that is to say the greater the slope of this curve). Moreover, the higher the number of cavities, the better the coefficient of attenuation outside the passband.

For the multifluorescence application, the excitation or emission spectra of the fluorochromes used are very close to each other. It is therefore necessary to recover the minimum amount of signal possible outside the passband. Accordingly, 6-cavity filters which offer the best characteristics at this level were chosen.

The filters used preferably have the following specifications:

- they are designed to be used at normal light incidence,
- the tolerance on the centre wavelength (λ_0) is $\pm$ 20% of the passband, for example, for a filter with a passband of 10 nm, λ_0 will be defined with a tolerance of $\pm$ 2 nm,
- the tolerance on the width of the passband is $\pm$ 20%,
- the coefficient of transmission T of these filters is greater than 50%,
- the out-of-passband rejection of these filters is 5OD for ultraviolet at 1200 nm; this means that outside the passband, the coefficient of transmission is 10^{-5}, that is to say 0.001%. For standard filters, the out-of-passband rejection

is ensured for wavelengths ranging from 0.8 λ_0 to 1.2 λ_0; for example, for a filter of $\lambda_\circ$ = 620 nm, the out-of-passband rejection occurs only between 500 and 740 nm. However, for the multifluorescence application, fluorochromes are observed whose spectra extend from 350 to 800 nm. Accordingly, filters were used whose out-of-passband rejection is ensured for ultraviolet at 1200 nm.

Finally, the present invention relates to a labeling kit characterized in that it comprises at least DNA probes as described above or a set of probes as mentioned above.

The present invention relates to a multicolor FISH diagnostic kit, characterized in that it comprises the DNA probes as described above or a set of DNA probes as mentioned above and a combination of filters and of fluorophores as described above.

The FISH or multi-FISH technique to which reference is or will be made several times in the present description is in particular described in Speicher et al., 1996; Schröck et al., 1996.

Other characteristics and advantages of the present invention will emerge on reading the examples below.

The combinations of fluorophores and of optical filters described in the invention may be used in multiple techniques involving fluorescence microscopy. Indeed, the fluorophores described in the present invention may be used to label many molecules or structures. Nonexhaustively, said fluorophores may be used to label polypeptides, antibodies, nucleic acids, phospholipids, fatty acids, sterol derivatives, membranes, organelles and many other biological macromolecules. The organelles may be mitochondria, endoplasmic reticulum, Golgi apparatus and lysosomes.

The combination of fluorophores and of optical filters according to the invention may be used to carry out FISH. In particular, this may allow the simultaneous use of several probes. This combination may be used to study multiple aspects such as cell morphology, the cytoskeleton, cell receptors, ion channels, neurotransmitters, the circulation of fluids, membrane fluidity, cell viability and proliferation, apoptosis, pinocytosis, endocytosis and exocytosis,

transduction, pH and ion concentrations (for example calcium, potassium, magnesium and zinc concentrations) (Richard P Haugland, 1996, Molecular Probes, MTZ Spend Ed.). It may allow the study of expression and translation.

The invention therefore also relates to a combination of fluorophores chosen from: fluorescein isothiocyanate (FITC), Texas Red (TR for Texas Red), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

Preferably, the combination of fluorophores comprises at least 2, 3, 4, 5, 6 or 7 fluorophores chosen from: fluorescein isothiocyanate (FITC), Texas Red (TR for Texas Red), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

A combination of preferred fluorophores of the present invention comprises the following 5 fluorophores: fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5) and cyanine 7 (Cy7).

Another preferred combination of fluorophores of the present invention comprises the following 6 fluorophores: fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), Bodipy 630/650, cyanine 5 (Cy5) and cyanine 7 (Cy7).

Another preferred combination of fluorophores of the present invention comprises the following 6 fluorophores: fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), Bodipy 630/650 and cyanine 5 (Cy5).

The combinations of fluorophores according to the invention may be included in a multicolor FISH diagnostic kit.

The combinations of fluorophores according to the invention may be used to label an entity chosen from polypeptides, antibodies, nucleic acids, phospholipids, fatty acids, sterol derivatives, membranes, organelles and biological macromolecules.

In addition, the invention relates to a combination of fluorophores combined with a pair of optical filters chosen from:

a. the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm combined with an excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical),

b. the fluorophore Cy3 having a maximum absorption wavelength of 554 nm

and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical),

c. the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical),

d. the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emission wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical),

e. the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical),

f. the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical),

g. the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm combined with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650DF10 type (Omega Optical).

Preferably, the combination of fluorophores combined with a pair of optical filters comprises at least 1, 2, 3, 4, 5, 6 or 7 fluorophores and filters chosen from:

a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm combined with an excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical),

b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10

type (Omega Optical),

c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical),

d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emission wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical),

e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical),

f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical),

g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm combined with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650DF10 type (Omega Optical).

The combinations of fluorophores combined with the pair of optical filters according to the invention may be included in a multicolor FISH diagnostic kit.

The combinations of fluorophores combined with a pair of optical filters according to the invention may be used to label an entity chosen from polypeptides, antibodies, nucleic acids, phospholipids, fatty acids, sterol derivatives, membranes, organelles and biological macromolecules.

The probes and combinations of fluorophores according to the invention may be used with any type of microscope (monochromator, laser, fluorescence microscope). Preferably, the invention uses a fluorescence microscope.

Various publications and patents are cited in the description. The disclosures contained in the publications and patents identified by references in this application

are incorporated by way of reference into the present application for a more detailed description of the content of the present invention.

EXAMPLES

1. Preparation of the probes

The genomic DNA extracted from various human-rodent somatic hybrid lines (NIGMS Human genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camdem) (Table 1) served as template for the PCR.

Table 1

Chromosome	Line reference
1	GM 13139
2	GM 10826B
3	GM 10253
4	GM 10115
5	GM 10114
6	GM 11580
7	GM 10791
8	GM 10156C
9	GM 10611
10	GM 10926B
11	GM10927A
12	GM 10868
13	GM 10898
14	GM 11535
15	GM 11715
16	GM 10567
17	GM 10498
18	GM 11010
19	GM 10449
20	GM 13260

Chromosome	Line reference
21	GM 10323
22	GM 10888
X	GM 6318B

The PCR was carried out either in the presence solely of the primer SR1 (situated at the 3' end of the Alu consensus sequence, position 241 to 261 : 5' CCACTGCACTCCAGCCTGGG 3' (SEQ ID No. 1) (Romana et al., 1993), or in the presence of the primer SR1 and of the primer L1H:

5' CATGGCACATGTATACATATGTAAC(A/T)AACC 3' (SEQ ID No. 2 and No. 3) (Ledbetter et al., 1990). When only the primer SR1 was used in the PCR, the product of amplification labeled and used as probe on metaphase chromosomes was stained almost completely the corresponding chromosome [sic] (with the exception of the centromeric regions) with an R-type band profile (as has been described by Lichter et al., 1990 with other types of Alu primers). However, in order to have a representation of the negative R bands, a PCR was also carried out by incorporating the 2 primers: SR1 and L1. Thus, when the 2 products of amplification (SR1 and SR1/L1) were mixed and used as probe, the negative R bands were indeed stained and the telomeric regions were perfectly delimited.

PCR conditions

The PCR reaction took place in a final volume of 50 µl containing 500 ng of genomic DNA (somatic hybrid), 1 µM of each oligonucleotide (either 1 µM SR1, or 1 µM SR1 and 1 µM L1H), 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin, 250 µM of each deoxynucleotide triphosphate (dATP, dGTP, dCTP, dTTP) and 2.5 U of *Thermophilus aquaticus* DNA polymerase (Perkin-Elmer-Cetus). The initial denaturation was carried out at 96°C for 6 min, followed by 30 cycles: denaturation at 94°C for 1 min, annealing at 63°C for 1 min, extension at 72°C for 10 min At the end of the cycles, a final extension at 72°C for 10 min was carried out.

The 2 products of amplification (SR1 and SR1/L1H) were mixed and then precipitated with ethanol. The DNA pellet was taken up in 20 µl of water and the DNA concentration was estimated on a 1.3% agarose gel.

Labeling of the probes by "Nick translation"

15 µg of the mixture of PCR products were able to be labeled during a single Nick translation reaction. The reaction took place in a final volume of 500 µl, containing 20 µM of each of the deoxynucleotide triphosphates (dATP, dGTP, dCTP), 10 µM of dTTP and 10 µM of modified dUTP, 50 µM Tris-HCl , 5 µM $MgCl_2$, 1 µM β-mercaptoethanol and 20 U of a mixture of enzymes (Dnase/Polymerase : Boehringer-Mannheim). To directly label the probe in fluorescence, the modified nucleotide was either dUTP-12-FITC (Boehringer-Mannheim) or dUTP-12-Texas Red (Molecular Probes) or dUTP-Cy3 (Amersham) or dUTP-Cy5 (Amersham). On the other hand, for an indirect labeling, dUTP-16-biotinylated (Boehringer-Mannheim) was used. The labeling was carried out in an Eppendorf tube (2 ml) overnight at 15-16°C. The free nucleotides were then removed by precipitation of the probe with ethanol. The DNA pellet was taken up in 500 µl of TE 10:1 (10 mM Tris-HCl, 1 mM EDTA, pH8) so that the probe is at a concentration of about 30 ng/µl.

Composition of the mixture of the 23 chromosome paint probes

Each probe specific for a chromosome was labeled individually with the various modified dUTPs (fluorescent or otherwise). Depending on the richness in R and G bands of each of the chromosomes and depending on their size, those which had or otherwise to be composed of different fluorochromes were established a priori. For example, combinations of 3 or 4 fluorochromes were preferably used for chromosomes rich in R bands (e.g.: chromosome 19); on the other hand, for chromosomes low in R bands, only 1 to 2 fluorochromes were used (e.g.: chromosome 8). This choice also depended, for each fluorochrome, on the combination of excitation and emission filters. Indeed, when a probe was labeled in equivalent proportion with different fluorochromes, the signal intensities for the different fluorochromes are not necessarily comparable. That depends indeed on the quality of the excitation and emission filters for each fluorochrome, but also on the quantity of fluorescence emitted by the fluorochrome. The latter itself depends on the intensity of the excitation luminous flux and therefore on the spectral power of the light source (Mercury Lamp HBO 100 W - OSRAM). Among all these parameters, it was preferably chosen to optimize the resolving power of the

combinations of filters, in order to obtain on emission the best signal/background noise ratio for each fluorochrome. Indeed, the differences in intensity of fluorescence may be compensated for, on the one hand, by increasing or decreasing the exposure time during the acquisition of the image by the camera (Hamamatsu C4880), but also by varying the concentrations of probes depending on the fluorescent marker used. Finally, the concentrations of probes were adjusted such that for a given fluorochrome (and therefore for a given filter combination), all the labeled chromosomes emit a fluorescence of equivalent intensity.

The composition of the 23 chromosome paint probes was therefore defined experimentally and precisely after many control experiments (Table 2).

400 µg (about 27-fold) of Cot1 DNA (human competitor DNA) were added to these 1470 ng of mixture of chromosomal probes (50 different probes). The DNA mixture was then precipitated with ethanol. The pellet was taken up in 10 µl of hybridization mixture (50% formamide, 10% dextran sulfate, 2 X SSC (pH 7), 1 µg/µl of sonicated herring sperm DNA).

Table 2

Fluor Chrom.	FITC	Cy3	TR	Cy5	Bio/Cy7	Mixture 1470 ng=
1		35				35
2			40			40
3	30				25	55
4				50		50
5	50					50
6				40	30	70
7			30		35	65
8	60			30		90
9	30	20				50
10		30	25		20	75
11		30		40		70
12			35	50		85
13	60		45			105
14	25	20			25	70
15	50	15	15			80
16	30	20		20		70
17		15		25	20	60
18					40	40
19	25	10	20		20	75
20		20	20	30		70
21		30	30			60
22	10		15	20		45
X		30			30	60

Full name of the fluorochromes:

FITC: Fluorescein isothiocyanate; TR: Texas Red; Cy3: Cyanine 3; Cy5: Cyanine 5; Bio: Biotin; Cy7: Cyanine 7

An alternative preparation of the probes

Alternatively, the preparation of the mixture of probes was carried out before their labeling by Nick translation for each fluorochrome (Table 3). 5 mixtures of probes were thus obtained which were labeled by Nick translation with the aid of modified nucleotides according to the following protocol.

1 to 2 µg of the mixture of probes were labeled by Nick translation in a volume of 50 µl containing: 20 µM of each of the deoxynucleotide triphosphates (dATP, dGTP, dCTP), 10 µM of dTTP and 10 µM of modified dUTP, 50 µM Tris-HCl , 5 µM $MgCl_2$, 1 µM β-mercaptoethanol and 20 U of a mixture of enzymes (Dnase/Polymerase : Boehringer-Mannheim). To directly label the probe in fluorescence, the modified nucleotide was dUTP-12-FITC (Boehringer-Mannheim) or dUTP-12-Texas Red (Molecular Probes) or dUTP-Cy3 (Amersham) or dUTP-Cy5 (Amersham). On the other hand, for an indirect labeling, dUTP-16-biotinylated (Boehringer-Mannheim) was used. The labeling was carried out in an Eppendorf tube (2 ml) overnight at 15-16°C.

After labeling, the 5 mixtures of chromosomal probes (14.75 µg) were precipitated together (with ethanol) in the presence of 400 µg (about 27-fold) of Cot1 DNA (human competitor DNA). The pellet was taken up in 10 µl of hybridization mixture (50% formamide, 10% dextran sulfate, 2 X SSC (pH 7), 1 µg/µl of sonicated herring sperm DNA).

This alternative method of labeling has the advantage of making the protocol for labeling the probes less cumbersome and simpler by reducing the number of labelings by Nick translation to five instead of fifty and by preparing a single mixture of probes instead of two.

2. Fluorescence *in situ* hybridization

The procedure was that described by Cherif et al., 1990, with a few modifications.

Preparation of the chromosomes in metaphase

The preparation of the metaphase chromosomes was carried out starting with a culture of circulating lymphocytes obtained by venous puncture of a normal subject. The lymphocytes stimulated by phytohemagglutinin (PHA) (100 µl per 8 ml of culture) were cultured for 72 hours at 37°C in RPMI-1640 medium. The

cells were then synchronized by adding methotrexate (10 μM) for 17 hours and then rinsed and recultured in the presence of 5-bromodeoxyuridine (BrdU) (0.1 mM) for 6 hours. After the action of colchicine (1 mg/ml) for 15 min, the disruption of the cells was obtained by resuspension in a hypotonic KCl solution (75 mM). The chromosomes were fixed in a methanol/acetic acid mixture (3 vol/1 vol) and one to two drops of cellular suspension were spread on each slide. The slides, dried at room temperature for 2 to 3 days, were then stored at -20°C for several months.

Table 3

Fluor. / Chrom.	FITC	Cy3	TR	Cy5	Bio/Cy7	Mixture
1		0.3				0.3
2			0.4			0.4
3	0.3				0.25	0.55
4				0.5		0.5
5	0.5					0.5
6				0.4	0.3	0.7
7			0.3		0.35	0.65
8	0.6			0.3		0.9
9	0.3	0.2				0.5
10		0.3	0.25		0.2	0.75
11		0.3		0.4		0.7
12			0.35	0.5		0.85
13	0.6		0.45			0.105
14	0.25	0.2			0.25	0.7
15	0.5	0.15	0.15			0.8
16	0.3	0.2		0.2		0.7
17		0.15		0.25	0.2	0.6
18					0.4	0.4
19	0.25	0.1	0.2		0.2	0.75
20		0.2	0.2	0.3		0.7
21		0.3	0.3			0.6
22	0.1		0.15	0.2		0.45
X		0.3			0.3	0.6
Total (µg)	3.7	2.7	2.75	3.15	2.45	14.75

Full name of the fluorochromes:

FITC: Fluorescein isothiocyanate; TR: Texas Red; Cy3: Cyanine 3; Cy5: Cyanine 5; Bio: Biotin; Cy7: Cyanine 7

Preparation of the slides

The slides were treated with RNase (100 µg/ml) for 1 hour at 37°C and then rinsed 3 times (5 min each) in a 2 X SSC solution, pH 7. The slides were then dehydrated by successive passes in a series of ethanol baths at increasing concentration (70%, 80%, 90% and 100% and 2 min per bath), and dried. The chromosomal DNA was denatured by immersing the slides in a 70% formamide/2 X SSC bath (pH 7) at 70°C for 2 minutes. The denaturation was stopped by immersing the slides (2 minutes) in 70% ethanol cooled to -20°C and kept in an ice bath. The slides were then dehydrated by successive passes in a series of ethanol baths at increasing concentration and dried. The slides were then incubated for 8 to 10 min at 37°C in a solution (20 mM Tris-HCl, pH 7.4, 2 mM $CaCl_2$) containing proteinase K (100 ng/ml) and then dehydrated in a series of ethanol baths and dried.

Hybridization and detection of the signal

The mixture of probes and of competitor DNA (10 µl) was denatured for 10 min at 70°C and then immersed in ice and prehybridized for at least 3 hours at 37°C. This mixture was then deposited on the slide and covered with a glass coverslip (18 X 22 mm). The hybridization took place over 2-3 days at 37°C in a humid chamber.

The slides were then washed in a series of 3 baths of 50% formamide, 2 X SSC, pH 7 (3 min each) at a temperature of 42-45°C, followed by 5 washes in 2 X SSC, pH 7 (2 minutes each) and by a one minute wash in a 1 X BN solution (0.1 M sodium bicarbonate, 0.05% nonidet P-40). The biotinylated probes were detected by adding avidin (Vector Laboratories Biosys) coupled to cyanine 7 (Amersham) (Avidin-Cy7) (5 µg/ml). The coupling of the avidin to cyanine 7 was carried out with a coupling kit (Amersham). To reduce the problems of background noise linked to nonspecific bindings of avidin, the slides were previously incubated for 10 minutes in a 1 X BN solution containing 5% skimmed milk powder. The slides were then incubated for 1/2 an hour at 37°C in a solution containing avidin-Cy7 (5 µg/ml in 1 X BN + 5% milk powder) and then successively washed 3 times (2 minutes) in a 1 X BN solution at 45°C. The fluorescent signal was amplified by adding a layer of biotinylated anti-avidin antibodies (5 µg/ml) (Vector

Laboratories, Biosys France), followed by a layer of avidin-Cy7 (5 µg/ml) according to the protocol described by Pinkel et al., 1986. For each layer, the slides were incubated for 30 minutes at 37°C and then washed 3 times in a 1 X BN solution. The probes labeled with dUTP-FITC, dUTP-TR, dUTP-Cy3 and dUTP-Cy5 required no additional revealing step.

The slides were examined with the aid of an epifluorescence photomicroscope (DMRX B, LEICA) equipped with the combination of filters which is described above. Two independent wheels were used to carry 8 filters each. The wheel carrying the excitation filters was inserted immediately after the Hg lamp and that carrying the emission filters was placed above the objectives and the beam separating filter. Before the acquisition of the images, 20 µl of an anti-fade solution (Johnson et al., 1981) were deposited on each slide and covered with a coverslip (anti-fade solution: 100 mg of PPD (p-phenylenediamine, Sigma) in a solution composed of 10 ml of PBS and 90 ml of glycerol; the pH of the solution was adjusted to 8.0 with 0.1 M NaOH). The anti-fade solution makes it possible to avoid the rapid extinction of the fluorescence emitted by the different fluorochromes when they are subjected to strong irradiation.

3. Example of use of a combination of 6 fluorophores

Since cyanine 7 is a relatively unstable fluorochrome, efforts were made to replace it with another fluorochrome, for example Bodipy 630/650 (Molecular Probes). Coupled to an antibody or a molecule of avidin, Bodipy makes it possible to indirectly reveal a probe labeled with biotin, with digoxigenin or with dinitrophenol (DNP). In this case, the choice of the 5 fluorochromes for the multifluorescence can no longer be the same because the absorption and emission spectra of cyanine 5 and of Bodipy 630/650 are too close for there to be good discrimination between these two fluorochromes. The choice will be the following:

- fluorescein isothiocyanate (FITC)
- Texas Red (TR)
- Cyanine 3 (Cy3)
- Bodipy 630/650
- Cyanine 5.5 (Cy5.5)

This choice also has the advantage of allowing the use of cyanine 7 as 6th fluorochrome if need be (for example for applications where it is not possible to carry out combinatory probe labeling, and where it would be advantageous to have different probes which can be discriminated between with a maximum of different fluorochromes).

In this case, the Cy5.5 should also be coupled to an antibody or a molecule of avidin in order to be able to reveal a probe labeled with biotin, digoxigenin or DNP.

For example, for multifluorescence karyotyping, the different systems for labeling and revealing the probes will be:

Labeling	Type of labeling	Revealing
fluorescein isothiocyanate (FITC)	direct	no
Texas Red (TR)	direct	no
Cyanine 3 (Cy3)	direct	no
digoxigenin (Dig)	indirect	anti-dig-Bodipy 630/650
biotin (Bio)	indirect	avidin-Cy5.5

Should it be desirable to use 6 fluorochromes at the same time, the choice would be:

Labeling	Type of labeling	Revealing
fluorescein isothiocyanate (FITC)	direct	no
Texas Red (TR)	direct	no
Cyanine 3 (Cy3)	direct	no
dinitrophenol (DNP)	indirect	anti-DNP Bodipy 630/650
digoxigenin (Dig)	indirect	anti-dig-Cy5.5
biotin (Bio)	indirect	avidin-Cy7

Although the preferred embodiments of the invention have been illustrated and described, it should be considered that many changes may be made by persons skilled in the art to depart [sic] from the spirit and scope of the present invention.

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CLAIMS

1) Probes intended for the labeling of a chromosome, characterized in that they are composed of a set of DNA segments which are more represented in certain chromosome bands and which are obtained by IRS-PCR amplification from said chromosomes with the aid of primers specific for the Alu and LINE DNA sequences.

2) Probes according to claim 1, characterized in that the DNA segments amplified by IRS-PCR have as source mono-chromosomal rodent-human somatic hybrids.

3) Probes according to either of claims 1 and 2, characterized in that said probes are specific for a human chromosome.

4) Probes according to one of claims 1 to 3, characterized in that the DNA segments are more represented in one type of cytogenetic bands.

5) Probes according to one of claims 1 to 4, characterized in that the DNA segments are more represented in the R bands.

6) Probes according to one of claims 1 to 5, characterized in that the specific primers used comprise or are primers having the sequences:

- SEQ ID No. 1 for the primer specific for the Alu DNA sequences,
- SEQ ID Nos. 2 and 3 for the primer specific for the LINE DNA sequences.

7) Probes according to one of claims 1 to 6, characterized in that the probes are derived from a mixture of two IRS-PCR amplification products which is composed of:

- PCR amplification product obtained using the primer specific for the Alu DNA sequences,

- PCR amplification product obtained using the primer specific for the Alu DNA sequences and the primer specific for the LINE DNA sequences.

8) Probes according to one of claims 1 to 7, characterized in that the DNA segments are labeled directly or indirectly by fluorescence techniques.

9) DNA probes according to claim 8, characterized in that the DNA segments are labeled with at least one fluorophore chosen from fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

10) Set of probes intended for labeling human chromosomes, characterized in that it contains probes according to one of claims 1 to 9 for each of the human chromosomes or for a number of them.

11) Method of producing probes intended for labeling human chromosomes, characterized in that said method comprises the mixing of two amplification products obtained by two IRS-PCR amplifications from said chromosomes using, on the one hand, PCR primers specific for the Alu and LINE DNA sequences, and, on the other hand, PCR primers specific for the Alu DNA sequences.

12) Probes intended for labeling human chromosomes obtained by a method according to claim 11.

13) Multicolor FISH method, characterized in that it uses for its implementation probes according to one of claims 1 to 9 or 12 or a set of probes according to claim 10.

14) Method according to claim 13, characterized in that the DNA probes are labeled with fluorophores and in that each fluorophore having a specific absorption and emission wavelength is combined with a pair of optical filters, one for absorption and one for emission, said method using fluorophores and pairs of

filters chosen from the following group:

a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm combined with an excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical),

b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical),

c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical),

d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emission wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical),

e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical),

f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical),

g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm combined with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650DF10 type (Omega Optical).

15) Method according to claim 14, characterized in that the optical filters exhibit the following qualities:

- they are of the 6-cavity type,

- they have an ADI of 0°;
- they have a tolerance $\lambda_0 \pm 20\%$ of FWHM,
- they have a tolerance on FWHM of $\pm$ 20% of FWHM,
- they have an OD5 out-of-passband rejection of UV at 1200 nm
- they have a transmission curve $T \geq 50\%$ at λ_0 .

16) Method according to claim 15, characterized in that the optical filters exhibit, in addition, the following characteristics:

- they have a centered useful diameter greater than 21 mm,
- they have a thickness $\leq$ 7 mm.

17) Method according to one of claims 13 to 16, characterized in that said probes are used for the study of karyotypes and of karyotypes of chromosome rearrangements.

18) Diagnostic kit characterized in that it comprises at least one of the DNA probes as described in claims 1 to 9 or 12 or a set of probes according to claim 10.

19) Kit according to claim 18, characterized in that the DNA probes are labeled with fluorophores and in that each fluorophore having a specific absorption and emission wavelength is combined with a pair of optical filters, one for absorption and one for emission, said kit using fluorophores and pairs of filters chosen from the following group:

a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm combined with an excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical),

b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical),

c) the fluorophore TR having a maximum absorption wavelength of 593 nm

and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical),

d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emission wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical),

e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical),

f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical),

g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm combined with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650DF10 type (Omega Optical).

20) Combination of fluorophores characterized in that it comprises at least 3 of the fluorophores are [sic] chosen from fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

21) Combination according to claim 20, characterized in that it comprises at least 4 of the fluorophores chosen from fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

22) [sic] Combination according to claim 20, characterized in that it comprises at least 5 of the fluorophores chosen from fluorescein isothiocyanate

(FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

22) Combination according to claim 20, characterized in that it comprises at least 6 of the fluorophores chosen from fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

23) Combination according to claim 20, characterized in that it comprises the following 5 fluorophores: fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 7 (Cy7).

24) Combination of fluorophores combined with a pair of optical filters, characterized in that it comprises at least 1 fluorophore combined with a pair of optical filters chosen from:

a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm is coupled with an excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical),

b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical),

c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical),

d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emission wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical),

e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm

and a maximum emission wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical),

f) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical),

g) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm combined with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650DF10 type (Omega Optical).

26
25) Composition according to one of claims 20 to 24, characterized in that it is included in a multicolor FISH diagnostic kit.

27
26) Combination according to one of claims 20 to 24, characterized in that it is used to label an entity chosen from polypeptides, antibodies, nucleic acids, phospholipids, fatty acids, sterol derivatives, membranes, organelles and biological macromolecules.

Descriptive abstract

The present invention relates to fluorescent probes which can be used in multicolor fluorescence *in situ* hybridization, and mainly chromosome painting. The probes intended for labeling a chromosome are such that they are composed of a set of DNA segments which are more represented in certain chromosome bands and which are obtained by IRS-PCR amplification from said chromosomes using PCR primers specific for the repeated and dispersed Alu and LINE DNA sequences.

The invention comprises, in addition, methods of producing said probes, multicolor FISH methods which can use said probes as well as diagnostic kits comprising them.

Finally, the invention comprises combinations of fluorophores and optical filters.

#7.

PTO/PCT Rec'd 23 AUG 2002

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PTO/SB/122 (10-00)
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CHANGE OF CORRESPONDENCE ADDRESS *Application*		
Address to: Assistant Commissioner for Patents Washington, D.C. 20231	**Application Number**	09/807,507
	Filing Date	April 13, 2001
	First Named Inventor	Dorra Cherif
	Group Art Unit	n/a
	Examiner Name	Unknown
	Attorney Docket Number	52.US2.PCT

Please change the Correspondence Address for the above-identified application to:

☐ Customer Number 23557 → Place Customer Number Bar Code Label Here

OR

☒ Firm *or* Individual Name	**Frank C. Eisenschenk, Ph.D.**				
Address	**SALIWANCHIK, LLOYD & SALIWANCHIK**				
Address	**2421 N.W. 41st Street, Suite A-1**				
City	**Gainesville**	State	**FL**	Zip	**32606-6669**
Country	**USA**				
Telephone	**(352) 375-8100**	Fax	**(352) 372-5800**		

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I am the:

☐ Applicant/Inventor.

☐ Assignee of record of the entire interest. (See 37 CFR 3.71. *Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)..*

☒ Attorney or Agent of record.

☐ Registered practitioner named in the application transmittal letter in an application without an executed oath or declaration. See 37 CFR 1.33(a)(1). Registration No. _____.

SIGNATURE of Applicant or Assignee of Record

Name	John Lucas, Ph.D., J.D.	Reg. No. 43,373
Signature	Aaron Eisenschenk REG.# P-52,193 (FOR JOHN LUCAS)	
Date	8/19/02	

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

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POWER OF ATTORNEY OR AUTHORIZATION OF AGENT

Page 1 of 1

Application Number	09/807,507
Filing Date	April 13, 2001
First Named Inventor	Dorra CHERIF
Group Art Unit	Unknown
Examiner Name	Unassigned
Attorney Docket Number	52.US2.PCT

I hereby appoint:

☐ Practitioners at Customer Number 23,557 →

OR

☒ Practitioner(s) named below:

Name	Registration Number
John M. Lucas	43,373
Peter Follette	46,213
Carol Johns	50,463
Aaron J.Scalia	P-52,193
Kristen K. Walker	P-52,335

Frank C. Eisenschenk; Reg. No. 45,332; and the registrants of the firm Saliwanchik, Lloyd & Saliwanchik, A Professional Association, 2421 N.W. 41st Street, Suite A-1, Gainesville, FL 32606-6669, Customer ID No. 23,557; as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please change the correspondence address for the above-identified application to:

☒ The above-mentioned Customer Number

OR

☒ Firm *or* Individual Name	Frank C. Eisenschenk, Ph.D.				
Address	Saliwanchik, Lloyd & Saliwanchik				
Address	2421 N. W. 41st Street, Suite A-1				
City	Gainesville	State	FL	Zip	32606-6669
Country	USA				
Telephone	(352) 375-8100	Fax	(352) 372-5800		

I am the:

☐ Applicant/Inventor.

☒ Assignee of record of the entire interest. (See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)..

SIGNATURE of Applicant or Assignee of Record

Name	John Lucas, Ph.D., J.D.
Signature	
Date	19 Aug 02

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their represenative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

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STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Dorra CHERIF

Application No./Patent No.: 09/807,507 Filed/Issue Date: April 13, 2001

Entitled: Fluorescent Probes for Chromosome Painting

GENSET, a corporation,
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. [X] the assignee of the entire right, title, and interest; or

2. [ ] an assignee of less than the entire right, title and interest.
The extent (by, percentage) of its ownership interest is _____%

in the patent application/patent identified above by virtue of either:

A. [x] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached. (Mailed for Recordation with the US PTO on May 24, 2002)

OR

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as shown below:

1. From:_____To:_____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame_____, or for which a copy thereof is attached.

2. From:_____To:_____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame_____, or for which a copy thereof is attached.

3. From:_____To:_____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame_____, or for which a copy thereof is attached.

[ ] Additional documents in the chain of title are listed on a supplemental sheet.

[ ] Copies of assignments or other documents in the chain of title are attached.
[NOTE: A separate copy *(i.e.,* the original assignment document or a true copy of the original document) must be submitted to Assignment Division in accordance with 37 CFR Part 3, if the assignment is to be recorded in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

19 Aug
Date

John Lucas, Ph.D., J.D.
Typed or printed name

John Lucas
Signature

Director, Intellectual Property
Title

Genset Corp.
10665 Sorrento Valley Road
San Diego, CA 92121
Phone: 858-597-2600 / Fax: 858-597-2601

ASSIGNMENT

WHEREAS, I, Dorra Cherif, a French citizen, residing at 11, rue Raymond Losserand, 75014 Paris, France, has invented certain new and useful improvements in a **Sondes Fluorescentes de Peinture Chromosomique**; for which I have filed an application for Letters Patent in the United States, Application Serial No. 09/807,507, filed on April 13, 2001.

AND WHEREAS, GENSET, S.A. (hereinafter "ASSIGNEE"), a French Corporation, with its principal place of business at 24, rue Royale, 75008 Paris, France, desires to acquire the entire right, title, and interest in and to the said improvements and the said Application:

NOW, THEREFORE, in consideration of the sum of One Dollar ($1.00) to me in hand paid, and other good and valuable consideration, the receipt of which is hereby acknowledged, I, the said inventor, do hereby acknowledge that I have sold, assigned, transferred and set over, and by these presents do hereby sell, assign, transfer and set over, unto the said ASSIGNEE, its successors, legal representatives and assigns, the entire right, title, and interest throughout the world in, to and under the said improvements, and the said application and all divisions, renewals and continuations thereof, and all Letters Patent of the United States which may be granted thereon and all reissues and extensions thereof, and all rights of priority under International Conventions and applications for Letters Patent which may hereafter be filed for said improvements in any country or countries foreign to the United States, and all Letters Patent which may be granted for said improvements in any country or countries foreign to the United States and all extensions, renewals and reissues thereof; and I hereby authorize and request the Commissioner of Patents of the United States, and any Official of any country or countries foreign to the United States, whose duty it is to issue patents on applications as aforesaid, to issue all Letters Patent for said improvements to the said ASSIGNEE, its successors, legal representatives and assigns, in accordance with the terms of this instrument.

AND I HEREBY covenant and agree that I will communicate to the said ASSIGNEE, successors, legal representatives and assigns, any facts known to me respecting said improvements, and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuing and reissue applications, make all rightful oaths and generally do everything possible to aid the said ASSIGNEE, its successors, legal representatives and assigns, to obtain and enforce proper patent protection for said improvements in all countries.

SIGNED this ____ day of 05/11/ 2001.

Dorra Cherif

Witnessed by: Name: NOUAK FREDERIQUE Signature:

Please Print or Type Name

09807507 .052902

#5

Please type a plus sign (+) inside this box → [+]

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POWER OF ATTORNEY OR AUTHORIZATION OF AGENT

Application Number	09/807,507
Filing Date	April 13, 2001
First Named Inventor	Dorra CHERIF
Group Art Unit	Unknown
Examiner Name	Unassigned
Attorney Docket Number	52.US3.PCT

I hereby appoint:

☐ Practitioners at Customer Number 000027206 → *Place Customer Number Bar Code Label here*

OR

☒ Practitioner(s) named below:

Name	Registration Number
John M. Lucas	43,373
Peter Follette	46,213
Lukas R. Voellmy	43,358

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please change the correspondence address for the above-identified application to:

☐ The above-mentioned Customer Number.

OR

☒ Firm *or* Individual Name	John Lucas, Ph.D., J.D.				
Address	Genset Corporation				
Address	10665 Sorrento Valley Road				
City	San Diego	State	CA	Zip	92121-1609
Country	USA				
Telephone	(858) 597-2600	Fax	(848) 597-2601		

I am the:

☒ Applicant/Inventor.

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

SIGNATURE of Applicant or Assignee of Record

Name	Dorra Cherif
Signature	
Date	05/11/2001

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing **OR** ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	52.US2.PCT
First Named Inventor	Dorra CHERIF
COMPLETE IF KNOWN	
Application Number	09 / 807,507
Filing Date	October 15, 1999
Group Art Unit	Unknown
Examiner Name	Unassigned

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Sondes Fluorescentes de Peinture Chromosomique

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 10/15/1999 as United States Application Number or PCT International Application Number 09/807.507 and was amended on (MM/DD/YYYY) 04/12/2001 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
PCT/FR99/02517	France	10/15/1999	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
		☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

Please type a plus sign (+) inside this box → [+]

PTO/SB/01 (10-00)
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DECLARATION — Utility or Design Patent Application

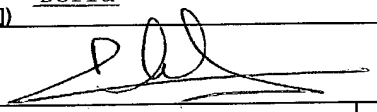
Direct all correspondence to: [X] Customer Number or Bar Code Label 000027206 OR [X] Correspondence address below

Name	John Lucas, Ph.D., J.D.
Address	Genset Corporation
Address	10665 Sorrento Valley Road
City	San Diego
State	CA
ZIP	92121-1609
Country	USA
Telephone	858/597-2600
Fax	858/597-2601

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR : ☐ A petition has been filed for this unsigned inventor

1 - 00

Given Name (first and middle [if any])	Dorra
Family Name or Surname	Cherif
Inventor's Signature	
Date	05/11/2001
Residence: City	Paris FRX
State	
Country	France
Citizenship	French
Mailing Address	24 rue Royale
Mailing Address	
City	Paris
State	
ZIP	75008
Country	France

NAME OF SECOND INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	
Family Name or Surname	
Inventor's Signature	
Date	
Residence: City	
State	
Country	
Citizenship	
Mailing Address	
Mailing Address	
City	
State	
ZIP	
Country	

☐ Additional inventors are being named on the ____supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

SEQUENCE LISTING

<110> Dorra CHERIF

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